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FAILURE TO OBTAIN POTENT SERUM FROM HORSE INJECTED WITH POLIOMYELITIC MATERIAL

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When so much is being done on the subject of immunity in epidemic poliomyelitis and so much more has yet to be learned concerning it, it may be well to record the negative results of an experiment in order that the same line of work may not be gone over again when it has proven by painstaking experiment to be of no value.

Early in 1917 it was determined to attempt immunization of a horse by injecting the filtrate of known poliomyelitic material under the skin, over a long period of time. Accordingly, injections were begun on March 10, 1917, with the unheated filtrate of a 1:50 emulsion of poliomyelitic brain and cord. This poliomyelitic material had been kept in 25% glycerol for about five months.

The horse received gradually increasing doses of this filtrate, beginning with 5 c c and working up to 120 c c at the end of a month, when the first bleeding was made. With the serum, Monkey 164 was injected in 5 c c doses as follows:

May 2: Under the skin morning and night; then again on May 3 and 4; 1 dose May 5, and 1 May 7; 2 doses on the 4 following days and 1 dose May 12.

May 7: Injected into right side of brain 1 c c emulsion of poliomyelitic cord from Monkey 155.

May 5-11: Moves slowly; unsteady.

May 12: Both legs flaccid; other members unaffected; nervous irritability.

May 13: Completely paralyzed in all members. Killed.

It was thus shown that the serum of the horse injected for six weeks with the unheated filtrate of poliomyelitic central nervous system was of no avail as a preventive against experimental poliomyelitis. The horse in the meantime had been receiving every few days steadily increasing doses of the poliomyelitic material, and after five months was bled again and the serum used in another attempt at immunization of monkeys against poliomyelitis.

MONKEY 166.—Injected subcutaneously with 5 c c morning and night on July 31.

Aug. 2: Not well, serum not given.

Aug. 3 and 4: Well, 10 c c under skin.

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Aug. 6: Injected intracerebrally with 1 c c of virus Monkey 164.

Aug. 7-10: 10 c c serum under skin daily. Well.

Aug. 11: Paralyzed in left arm, loss of power in right leg. Injected 3 c c serum into spinal canal after withdrawing 1.5 c c spinal fluid. This was followed by pressure symptoms and weakness in legs, so that animal could not sit up or walk; staring eyes; recovered equilibrium in about 10 minutes.

Aug. 12: Complete paralysis in all members. No treatment.

Aug. 13: Died last night.

Monkey 167.—July 31. Injected subcutaneously 5 c c, morning and night, serum of horse immunized with unheated filtrate poliomyelitic material.

Aug. 2: Not well, no serum treatment.

Aug. 3 and 4: Well, 10 c c under skin daily.

Aug. 6: Injected intracerebrally with 1 c c of virus Monkey 164.

Aug. 7, 8, 9: 10 c c serum under skin daily. Well.

Aug. 10: Hair ruffled; 8 c c serum injected into left femoral vein under ether.

Aug. 11: Animal shaky, with nervous twitching of head and limbs; 3 c c of serum injected into spinal canal, 2 minutes after which pressure symptoms occurred as manifested by rapid breathing, staring, shifting eyes, extreme weakness of legs and arms. Recovered in 5 minutes.

Aug. 12: Complete paralysis in all members. No treatment.

Aug. 13: Killed.

Monkey 168.—July 31. Received subcutaneously 5 c c, morning and evening, serum of horse immunized with filtrate of poliomyelitic material.

Aug. 3 and 4: 10 c c under skin in 2 places on each day.

Aug. 6: 1 c c virus Monkey 164 intracerebrally.

Aug. 7, 8, 9: 10 c c horse serum subcutaneously each day.

Aug. 8: 1 c c spinal fluid withdrawn and 2 c c horse serum injected.

Aug. 11: Animal seems slightly slow in movement, otherwise well, 2.5 c c horse serum into spinal canal. No pressure symptoms.

Aug. 12: Animal somewhat weak but no paralysis. Injected 3 c c horse serum into spinal canal. Pressure symptoms occurred but disappeared in about half an hour and monkey was able to climb cage.

Aug. 13: Animal completely paralyzed. Died at noon.

The experiments with 3 monkeys show that antibodies of sufficient potency to protect against experimental poliomyelitis had not developed in the serum of a horse injected over a period of five months with poliomyelitic material.

Finally a trial was made of the serum of the horse after seven months injection with poliomyelitic material. Two weeks prior to intracerebral inoculation with the virus, 10 c c serum were given subcutaneously daily for a week, then 5 c c subcutaneously and 2 c c intraspinally each day for a week.

This treatment was carried out in every detail, but notwithstanding this, the result in Monkey 176 proved that the horse serum contained no immunizing bodies after it had received injections of the filtrate of unheated poliomyelitic material for seven months.

MONKEY 176.—The animal was injected under the skin with 10 c c horse serum Nov. 26; Nov. 27, 28, 30, Dec. 1 and 3 with 5 c c twice daily.

Dec. 4: Began intraspinal injection of horse serum 2 c c, giving also 5 c c under the skin. This was continued on 5 successive days. On Dec. 10 the animal was injected intracerebrally with 0.5 c c emulsion of cord of Monkey 172. At same time 2 c c of horse serum was injected into spinal canal.

Dec. 12, 13, 14: 2 c c horse serum into spinal canal, 5 c c under skin each day

Dec. 15: Irritable since yesterday; flaccid paralysis of legs. No treatment.

Dec. 17: Moribund; killed.

The serum of the horse obtained after 7 months of injections was used also as a curative agent after infection, but before the first symptoms of poliomyelitis such as ruffling of the hair and nervous irritability manifested themselves. Five animals and a control were used in this experiment.

MONKEY 170.—This animal received into the brain 0.5 c c emulsion of cord and pons of Monkey 168 and 2 c c of horse serum intraspinally on Nov. 19.

Nov. 20: 2 c c horse serum into spinal canal, 5 c c under skin.

Nov. 21: 2 c c horse serum into spinal canal, 5 c c into femoral vein.

Nov. 22: 2 c c horse serum into spinal canal, 5 c c under skin.

Nov. 23: 2 c c horse serum into spinal canal, 5 c c under skin.

Nov. 24: 2 c c into spinal canal, 5 c c under skin.

Nov. 25: Paralyzed in arms, legs, back and neck muscles.

Nov. 26: Moribund; killed.

MONKEY 171.—Received the same treatment as Monkey 170 on Nov. 19.

Nov. 20: 2 c c horse serum into spinal canal, 5 c c under skin.

Nov. 21: 2 c c horse serum into spinal canal, 5 c c into femoral vein.

Nov. 23: 2 c c horse serum into spinal canal, 5 c c under skin.

Nov. 24: A little shaky, otherwise active and well; 2 c c horse serum into spinal canal, 5 c c under skin.

Nov. 25: Down completely; muscles of arms, legs, back and neck show flaccid paralysis.

Nov. 26: Dead.

MONKEY 172.—Control. Received 0.5 c c emulsion of cord and pons of Monkey 168, Nov. 19.

Nov. 23: Slow in movement, rather shaky.

Nov. 24: Paralyzed completely in legs; back muscles weak; arms not affected.

Nov. 25: Paralyzed in arms, back, neck and legs, moribund.

Nov. 26: Dead.

MONKEY 173.—Received same treatment as 172 on Nov. 19.

Nov. 22: Received 2 c c horse serum into spinal canal, 5 c c under skin.

Nov. 23: 2 c c horse serum into spinal canal, 5 c c into femoral vein, also on Nov. 24.

Nov. 25: Down on all fours. Paralyzed.

Nov. 26: Dead.

MONKEY 174.—Received same treatment as Monkey 172 on Nov. 19.

Nov. 22: 2 c c horse serum into spinal canal, 5 c c subcutaneously.

Nov. 23: 2 c c horse serum into spinal canal, 5 c c into vein.

Nov. 24: Very shaky, 2 c c horse serum into spinal canal, 5 c c subcutaneously.
Nov. 25: Paralyzed in all members; neck and back paralyzed.
Nov. 26: Dead.

MONKEY 175.—Received same treatment as Monkey 172, Nov. 19.
Nov. 22: 2 c c horse serum into spinal canal, 5 c c under skin.
Nov. 23: 2 c c horse serum into spinal canal, 5 c c serum into vein.
Nov. 24: Lies on bottom of cage, almost moribund. Killed.

The experiment with six monkeys shows the serum to have no curative value.

Microscopic examination of sections of the cords of all the monkeys that died showed typical lesions of epidemic poliomyelitis.

Neutralization experiments on monkeys with 0.5 c c of salt solution emulsion of the virus, 1 to 10, incubated at 38 C. for one-half hour with 1 c c of the serum of the 7 months' immunized horse, showed the serum to have no neutralizing value, the treated monkeys becoming paralyzed as soon as the control.

The reactive response of the horse to the injections of virus, thus seemingly evidencing the activity of the latter as an infective agent, was shown by a rise in temperature of from 1-5 degrees, after each injection; general muscular tremors, after many of the injections; and in one instance, after the injection of 120 c c of filtrate, the animal fell down, pulse became rapid, respiration labored, mucous membranes congested, and was very nervous and hypersensitive to sudden noises of any kind. These general reactions were probably anaphylactic in nature, and may not have been due at all to the specific effect of the poliomyelitis virus as such, but the exact nature cannot be known.

The horse began treatment by receiving 1 c c of the filtrate, and at the end of the 7th month when the last test of the potency of its serum was made on monkeys, was receiving 500 c c of the filtrate subcutaneously.